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Role of divalent cations on DNA polymorphism under low ionic strength conditions

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ABSTRACT

We have examined the conformational properties of poly(dG-m^5dC) under a variety of low salt conditions and sample preparations. Extensive dialysis against 0.5 mM Na-cacodylate resulted in a left-handed polynucleotide conformation as determined by circular dichroism, in agreement with recently reported results. Similarly, extensive dialysis against Tris-EGTA also led to a left-handed conformation. Dilution of these samples led to a transition to the right-handed conformation. More stringent treatments such as dialysis followed by passage over an ion exchange column also resulted in a right-handed conformation. When these various solutions were examined using atomic absorption spectroscopy, significant levels of Mg^{+2} were observed (> 190 per 1000 nucleotides) in all samples showing a left-handed form, while much lower levels (< 45 per 1000 nucleotides) were found in the low salt samples displaying a right-handed conformation. Addition of $MgCl_2$ to samples in which divalent cations had been almost completely removed led to the reformation of the left-handed form. These results indicate that the left-handed form seen under certain low salt conditions is due to the presence of Mg^{+2} ions that remain bound to the polynucleotide, even in the presence of EDTA.

INTRODUCTION

The left-handed, Z conformation of DNA has received wide attention since the X-ray crystallographic analyses of $(dC\text{-}dG)_3$ and other oligonucleotides provided a high resolution structure for this conformation (1-3). This left-handed form is typically stabilized by the presence of high concentrations of monovalent salt, e.g., 4.4 M NaCl, or lower concentrations of multivalent cations (4,5) as well as nonaqueous solvents such as ethanol (6). Behe and Felsenfeld (5) showed that methylation of cytosine at the 5 position significantly facilitates the conversion to the left-handed form by lowering the concentration of added cations needed to produce the B to Z transition. The literature in this area has been reviewed by Jovin et al. (7) and Rich et al. (8).

Recently, there have been several reports from various groups, including one from this laboratory, describing a low salt, left-handed form of

poly(dG-m^5dC) which was stable in the absence of any added oligovalent cations and which could be converted to the right-handed conformation by addition of NaCl at concentrations in the range of 2-30 mM (9-12). In the experiments described below, we demonstrate that this low salt, left-handed form appears to be stabilized by low levels of cations present in the polynucleotide preparation obtained from the supplier. When such cations have been completely or almost completely removed, as determined by atomic absorption spectroscopy, the polynucleotide takes on a right-handed, B conformation.

MATERIALS AND METHODS

Poly(dG-m^5dC) was obtained from Pharmacia P.L. Biochemicals. Sodium chloride, sodium cacodylate and disodium salt of ethylenediaminetetraacetic acid (EDTA; ACS grade) were obtained from Aldrich Chemical Company and used as such. Ethylene glycol-bis (β-amino ethyl ether) N,N'-tetracetic acid (EGTA) was purchased from Sigma Chemical Company while Tris (hydroxymethyl) amino methane (Tris) base (AR grade) was obtained from Mallinckrodt Inc. Dialysis tubing (1000 and 10000 molecular weight cut off), purchased from Spectrum Medical Industries, was washed thoroughly with deionized water (Barnstead, ~18.0 x 10^6 ohms) and equilibrated in the buffer used for dialysis for an hour before dialysis. Deionized water was used for all experiments. Plasticware was used wherever necessary. DNA concentration was determined spectrophotometrically using ε_{260}= 7100.

CD (circular dichroism) spectra were recorded on a Jasco J500A spectropolarimeter in Prof. J.T. Yang's laboratory, UCSF. For the temperature studies, a circulating water bath was used. The temperature of the samples was measured using a thermocouple placed directly in the sample chamber. The transitions were followed using changes in molar ellipticity values at 252 and 292 nm.

UV spectra were recorded on a Cary 118 spectrophotometer and a Gilford 2600 automated spectrophotometer. For the temperature studies, a Gilford 2327 thermoprogrammer was used. Experiments were carried out in microcuvettes (0.3 mL volume, 1 cm pathlength) and samples were maintained at each temperature for atleast $\frac{1}{2}$ hour before measurements were taken. The Z to B transitions were followed using changes in A_{295}/A_{260} ratios. The absorbance ratios were typically 0.40-0.45 for Z type and 0.25-0.30 for B type. Thermal transitions were monitored by following changes in A_{260} values.

Atomic absorption experiments were conducted at the Microanalytical Laboratory in the University of California at Berkeley using a Perkin Elmer

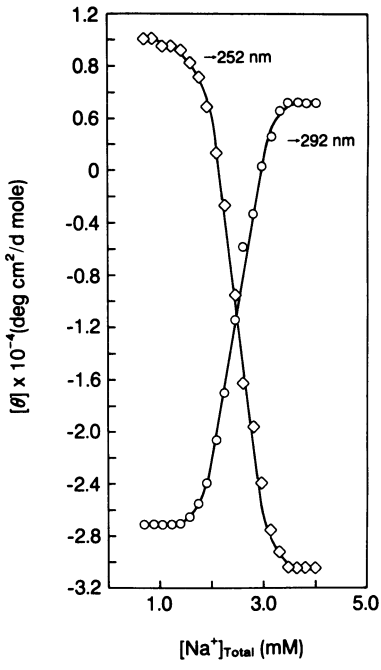

Figure 1. Change of ellipticity values at 252 and 292 nm with NaCl salt addition to poly(dG-m^5dC) in 0.5 mM Na-cacodylate, 0.1 mM Na_2 EDTA (undialyzed) at 25°C. The midpoint of transition occurs at ~ 2.4 mM total Na^+. DNA concentration is 153 μM nucleotide.

(model # 360) Atomic Absorption spectrophotometer.

AG50W-X8 cation exchange resin was obtained from Bio-Rad chemical division. It was packed in a 2 ml column and was regenerated by passing 2 L of 0.05 N HCl. The column was converted to Na^+ by passing through 1 L of 100 mM NaCl followed by 1 L of 0.5 mM sodium cacodylate buffer. 0.5 mM sodium cacodylate was found adequate to hold the pH of deionized water, which drops in pH on exposure to air, close to 7. A combination of 0.5 mM Tris base and 0.1 mM EGTA was used as a buffer containing a chelating agent but no sodium, with a pH of 7.5.

All experiments were carried out at 25°C unless otherwise specified.

RESULTS

Effect of NaCl addition and temperature on the undialyzed sample. Figure 1 shows the effect of NaCl addition to an undialyzed solution of poly(dG-m^5dC) in 0.5 mM sodium cacodylate and 0.1 mM Na_2 EDTA on the ellipticity values at 252 and 292 nm. Before salt addition, the undialyzed polymer is in a left-

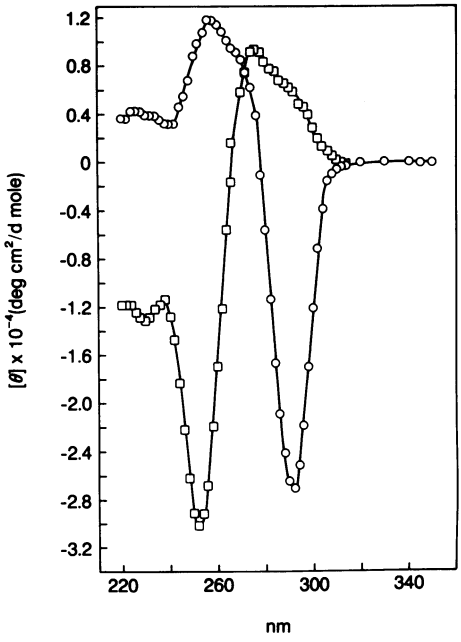

Figure 2. CD curves obtained before (o-o) and after (□ - □) NaCl addition experiment, for the same sample as in Figure 1. Amount of NaCl that has been added at the end of the titration is ~ 3.3 mM.

handed conformation at this DNA concentration (153 μM nucleotide). With salt addition, the polymer undergoes a Z → B transition, with the transition midpoint at ~2.4 mM total $[\overset{+}{Na}]$. Figure 2 shows the CD curves obtained before and after the NaCl addition experiment.

Dilution effect. Upon dilution of the undialyzed sample with buffer (0.5 mM sodium cacodylate and 0.1 mM Na_2EDTA), the polymer undergoes a transition from Z to B. Figure 3 shows the changes in A_{295}/A_{260} ratio values as a function of DNA concentration. The transition midpoint is around 16 μM nucleotide. The corresponding molar ellipticity changes shown in the same figure compare well with the absorbance ratio changes. Similar dilution experiments conducted on the undialyzed polymer with either just deionized water or 0.5 mM sodium cacodylate buffer did not cause any change in the ratio of absorbance values down to about 2 μM polynucleotide concentration. Thus, EDTA seems to have a role in the dilution effect.

NaCl addition experiments and effect of DNA concentration. Figure 4 shows the effect of NaCl addition on the absorbance ratio values at various DNA concentrations for the sample that has been dialyzed against 0.5 mM sodium

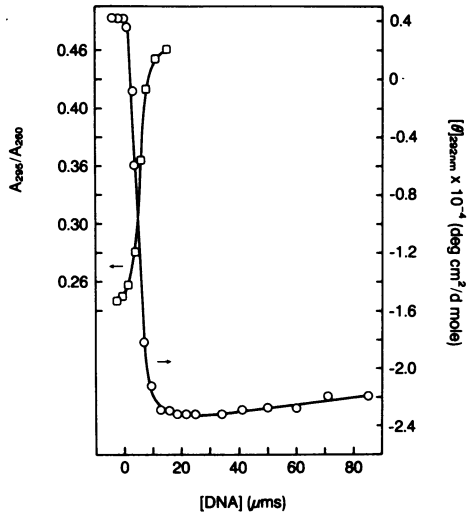

Figure 3. Changes in A_{295}/A_{260} values (□ - □) as a function of DNA concentration in same buffer as Figure 1. The midpoint of the transition is ~ 16 μm nucleotide. Also represented are the changes in ellipticity values at 292 nm (o-o) as a function of DNA concentration. The midpoint is ~ 15 μm nucleotide.

cacodylate only. A systematic decrease can be seen in the amount of NaCl needed to cause Z → B as the DNA concentration is decreased. Apparently the amount of NaCl needed to cause Z → B is much lower (for comparable DNA concentrations) if the chelator is present in the system, as can be seen by

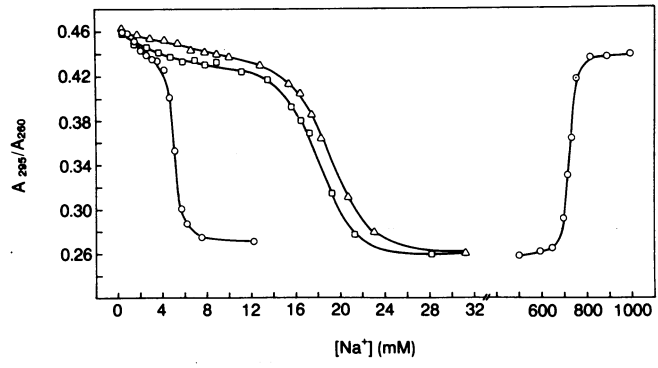

Figure 4. Changes in A_{295}/A_{260} values at different DNA concentrations as a function of Na^+ (total) concentrations for polymer that has been dialyzed against 0.5 mM sodium cacodylate only. ▲ - ▲ = 151 μm; □ - □ = 74 μm; o-o = 5 μm. The DNA concentrations are expressed in mole nucleotide.

TABLE 1. Number of Mg^{++} and Ca^{++} ions present per 1000 nucleotides in the poly(dG-m^5dC) samples at various stages of dialysis.

#	Solution	Conformation	Mg^{++}	Ca^{++}
1	Undialyzed polymer in 0.5 mM sodium cacodylate	Z	404	322
2	Polymer dialyzed against 0.5 mM sodium cacodylate	Z	195	72
3	Polymer dialyzed against 0.1 mM EGTA/0.5 mM Tris	Z	308	213
4	Polymer to which has been added 20 mM NaCl, then dialyzed against 0.5 mM sodium cacodylate	B	45	62
5	Polymer dialyzed first against (0.5 mM sodium cacodylate and 0.1 mM Na_2EDTA) and then 0.5 mM sodium cacodylate	B	25	5
6	Polymer dialyzed first against 1M NaCl, 0.5 mM sodium cacodylate + 0.1 mM Na_2 EDTA, next against 0.5 mM sodium cacodylate and then passed through AG50W-X8 column preequilibrated with 0.5 mM sodium cacodylate.	B	2	5

comparing Figures 1 and 4. Also shown in Figure 4 for one of the DNA concentrations is the B → Z transition observed at higher NaCl concentration (5).

Effect of divalent cations. The transition from a left-handed form to a right-handed form with increasing dilution of the polynucleotide shown in Figure 3 and the effect of poly(dG-m^5dC) concentration on the NaCl-induced transition shown in Figure 4 provoked concern that some trace amounts of cations may be present in the sample as supplied by the manufacturer. This problem was approached experimentally by determining the levels of two ubiquitous divalent cations, Mg^{+2} and Ca^{+2}, with atomic absorption spectroscopy. The results are summarized in Table 1. (In our discussion on the effect of divalent cations, we follow only Mg^{+2} for the purpose of clarity.) The undialyzed sample of poly(dG-m^5dC) dissolved in 0.5 mM Na-cacodylate showed a rather high ratio of about 400 Mg^{+2} ions per 1000 nucleotides. Extensive dialysis of this solution against 0.5 mM Na-cacodylate buffer alone (3 days, 3 changes of buffer, 2 ml solution against 2 L buffer) reduced this level by about 50%, and still resulted in a left-handed poly(dG-m^5dC) conformation. Extensive dialysis against a buffer consisting of tris base and EGTA (no Na^+) similarly led to a left-handed conformation and a

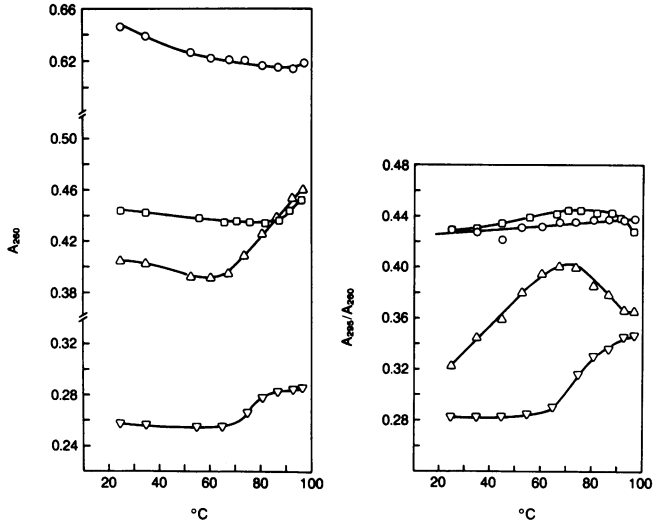

Figure 5. Changes in A_{260} and A_{295}/A_{260} values with temperature: o-o undialyzed sample in 0.5 mM sodium cacodylate; □ - □ dialyzed against 0.5 mM sodium cacodylate only; ▲ - ▲ dialyzed against 0.5 mM sodium cacodylate following addition of 20 mM NaCl; ▼ - ▼ solution that has been dialyzed extensively against 0.5 mM sodium cacodylate 0.1 mM Na_2EDTA etc and passed through AG50w-X8 column preequilibrated with 0.5 mM sodium cacodylate.

correspondingly high level of Mg^{+2}.

However, when 20 mM NaCl was added to the polynucleotide dissolved in 0.5 mM Na-cacodylate, which was then dialyzed against 0.5 mM Na-cacodylate, the resulting concentration of Mg^{+2} was reduced to 45 per 1000 nucleotides and the polynucleotide conformation under these conditions was right-handed. Similarly, dialysis against 0.5 mM Na-Cacodylate/0.1 mM Na_2 EDTA followed by dialysis against 0.5 mM Na-cacodylate alone reduced the Mg^{+2} level to 25 per 1000 nucleotides. Finally, when the polynucleotide solution was passed over a AG50W-X8 column that had been pre-equilibrated with 2 L of 0.5 mM Na-cacodylate, the concentration of cations was reduced to almost zero.

Melting studies. Figure 5 shows the changes in A_{260} and the absorbance ratio (A_{295}/A_{260}) values with temperature for (i) the undialyzed sample which is in Z form at room temperature, (ii) the sample that has been dialyzed against 0.5 mM sodium cacodylate only, which is also in Z form, (iii) the sample to which ~ 20 mM NaCl was added before dialyzing against 0.5 mM sodium cacodylate, which is in B form and (iv) the sample that has been dialyzed against 1M NaCl, then 0.5 mM sodium cacodylate/0.1 mM Na_2EDTA and then against

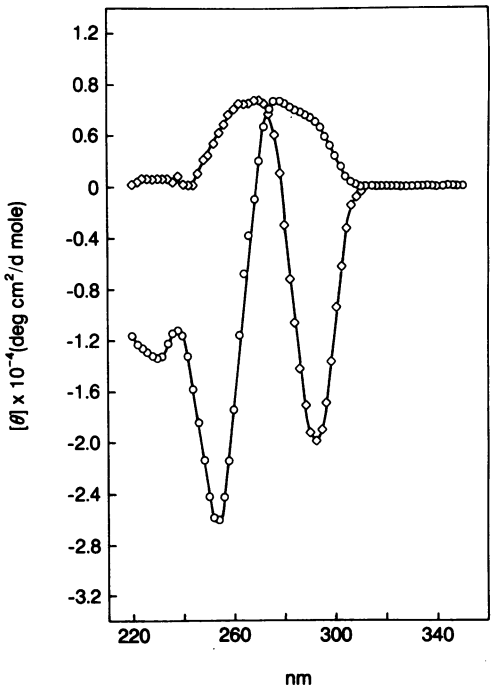

Figure 6. CD spectrum of poly(dG-m^5dC) in 0.5 mM sodium cacodylate solution that has been: (a) depleted of cations by dialysis and ion-exchange column chromatography, and (b) depleted of cations as in (a) and then to which 67 μm $MgCl_2$ has been added. DNA concentration is 52 μm in (a) and 48 μm in (b). The DNA concentrations are expressed in mole nucleotide. (a) is o-o. (b) is ◊ - ◊.

0.5 mM sodium cacodylate before finally passing it through a column of AG50W-X8 that has been preequilibrated with 2L of 0.5 mM sodium cacodylate, which is in B form. An increase in the A_{295}/A_{260} values without a corresponding increase in A_{260} values, indicates a B → Z transition, while an increase in the A_{260} values indicates melting.

With increasing temperature, the undialyzed polymer stays in Z form and does not melt. The second sample which has been dialyzed against 0.5 mM sodium cacodylate shows a tendency to melt. The third sample, which has gone to B on dialysis against 0.5 mM sodium cacodylate only after addition of ~ 20 mM NaCl, shows a B → Z transition before melting. The fourth sample which has been extensively dialyzed in the presence of a large excess of NaCl in the presence of 0.1 mM Na_2EDTA and subsequently passed through a column stays in B form with temperature before melting. The progressive removal of

the divalent cations by dialysis, etc. and the corresponding decrease in stability of the Z form can be easily seen by comparing these results with those from atomic absorption studies (Table 1).

Addition of Mg^{+2} to cation-depleted sample. As described above, upon depletion of divalent cations, the conformation of poly(dG-m^5dC) in low salt is right-handed. The CD spectrum of the polynucleotide under these conditions is displayed in Figure 6. Addition of $MgCl_2$ to this solution leads to an inversion of the CD spectrum and hence a conversion from the right-handed to the left-handed form (Figure 6). These results are in agreement with those previously reported by Chen et al. (13).

DISCUSSION

It is known that divalent cations even at low metal ion to DNA phosphate ratios can stabilize the Z form (13). It is also known that the amount of divalent cation needed to stabilize the Z form becomes less as the monovalent cation concentration is lowered (13). We find that the commercially supplied polymer still contains some residual divalent cations presumably carried over from the enzymatic synthesis. Addition of 0.1 mM Na_2EDTA to a solution of poly(dG-m^5dC) in 0.5 mM sodium cacodylate buffer does not remove enough of this divalent cation at higher DNA concentrations to promote the B conformation. Clearly the divalent cations are bound very tightly to the polynucleotide and remain so in the presence of EDTA. However, as the DNA concentration is lowered by dilution with buffer containing the chelator, the concentration of the divalent cations in the system is lowered, facilitating removal by the chelator of enough divalent cations to favor the B form. This explains the transition from Z to B as the DNA concentration is lowered at constant chelator concentration (Figure 3). Dilution with just the cacodylate buffer or the deionized water does not lead to a conformational transition.

NaCl addition experiments show that the amount of NaCl needed to the cause Z → B transition, at comparable DNA concentrations, is much higher in the absence of a chelator than in its presence (Figures 1 and 4) confirming the important role of both Na^+ and chelator in converting the divalent-cation-stabilized Z form to B form. Also when the DNA concentration is lowered, the amount of NaCl needed to cause Z → B is lowered (Figure 4) because of the lowering of the concentration of the divalent cations that stabilize the Z form.

It becomes clear from the above discussion that poly(dG-m^5dC) in low (monovalent) salt solution is actually in the right-handed B form and that the

presence of dialyzable trace amounts of divalent cations can stabilize the DNA in the Z form. Previous reports of a low salt, left-handed form of poly(dG-m^5dC) have involved samples obtained from the same supplier used in this study. Narasimhan and Bryan (9) worked with undialyzed samples of unspecified polynucleotide concentyration. The results of Krueger and Prairie (10) showed Z form for an undialyzed sample (100 μM in nucleotide) in the presence of 80 μM of EDTA. As demonstrated by our dilution experiments, the undialyzed polymer will be in Z form even in the presence of 100 μM EDTA and 500 μM sodium cacodylate at this DNA concentration. When Krueger and Prairie dialyzed a sample containing 30 mM NaCl against 1 mM NaCl, they observed a left-handed form (10). Our studies indicate that dialysis against low NaCl concentrations like 1 mM can remove divalent cations only partially and further treatment is needed for complete divalent cation removal.

Latha and Brahmachari (11) carried out their studies on samples dialyzed against 0.5 mM sodium cacodylate only. Again, even extensive dialysis against this low Na^+ concentration, in the absence of a chelator, does not remove the trace divalent cations that stabilize the Z form, as shown by the experiments presented above. Finally, in the study by Feuerstein et al. (12) on the low salt, left-handed form of poly(dG-m^5dC), dialysis was carried out under several different conditions, e.g. 2 M NaCl, 1 mM Na_2 EDTA. One must conclude that, despite this, not all the divalent cations were removed in those samples.

In summary, we have shown that the low salt, left-handed form of poly(dG-m^5dC) appears to be stabilized by trace amounts of divalent cations present in the sample as supplied by the manufacturer. When it can be demonstrated that such cations have been removed, and that only monovalent sodium is present, the polynucleotide adopts a right-handed conformation at low sodium concentrations. It is not possible to attribute the stability of the low salt, left-handed conformation in the undialyzed samples to the presence of Mg^{+2} alone. We did observe the presence of Ca^{+2} also. There may well be additional cations present which were not tested for and hence not detected. However, the observation of a left-handed conformation in cation-depleted samples to which $MgCl_2$ has been added indicates at least an important role for Mg^{+2} in this regard.

ACKNOWLEDGMENTS

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